

Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Currently Amended) A purified α -isomaltosylglucosaccharide- forming enzyme, which forms a saccharide, having a glucose polymerization degree of at least three and having both the α -1,6 glucosidic linkage as a linkage at the non-reducing end and the α -1,4 glucosidic linkage other than the linkage at the non-reducing end, by catalyzing the α -glucosyl-transfer from a saccharide, having a glucose polymerization degree of at least two and having the α -glucosidic linkage as a linkage at the non-reducing end; said enzyme being obtained from a microorganism of the genus *Bacillus* selected from the group consisting of *Bacillus globisporus* C9, FERM BP-7143, and mutants thereof and having the following physicochemical properties:

- a. Molecular weight about $140,000 \pm 20,000$, ~~or~~ $137,000 \pm 20,000$ -Daltons on SDS-PAGE;
- b. Isoelectric point (pI) about 5.2 ± 0.5 ~~or 7.3 ± 0.5~~ on isoelectrophoresis using ampholine;
- c. Optimum temperature
 - (i). About ~~40, 45, or~~ 50°C when incubated at a pH of 6.0 for 60 minutes;
 - (ii). About ~~45, 50, or~~ 55°C when incubated at a pH of 6.0 for 60 minutes in the presence of 1 mM Ca^{2+} ;
- d. Optimum pH about 6.0 to 6.5 when incubated at 35°C for 60 minutes;
- e. Thermal stability

- (i). Stable up to a temperature of about 35~~7~~
40, ~~or 45~~°C when incubated at a pH of 6.0
for 60 minutes;
- (ii). Stable up to a temperature of about 40~~7~~
45, ~~or 50~~°C when incubated at a pH of 6.0
for 60 minutes in the presence of 1 mM
Ca²⁺; and
- f. pH stability stable at pHs of about 4.5 to 9.0~~7~~
5.0 to 10.0 ~~or 5.0 to 9.0~~ when incubated at 4°C
for 24 hours.

2. (Cancelled)

3. (Currently Amended) The purified α -
isomaltosylglucosaccharide-forming enzyme of claim 1, ~~or 48, 52,~~
or 53 wherein said saccharide, having a glucose polymerization
degree of at least two and having the α -1, 4 glucosidic linkage
as a linkage at the non-reducing end, is one or more members
selected from the group consisting of maltooligosaccharides,
maltodextrins, amyloextrins, amyloses, amylopectins, soluble
starches, liquefied starches, and glycogens.

Claims 4-7. (Cancelled)

8. (Currently Amended) A process for producing the
purified α -isomaltosylglucosaccharide-forming enzyme of claim 1,
~~or 48, 52, or 53~~ which comprises:

- a. culturing in a nutrient culture medium a
microorganism capable of producing said
enzyme;
- b. and collecting said enzyme from the resulting
culture.

9. (Original) The process of claim 8, wherein said microorganism is of the genus *Bacillus* or *Arthrobacter*.

10. (Original) The process of claim 9, wherein said microorganism of the genus *Bacillus* is one selected from the group consisting of *Bacillus globisporus* C9, FERM BP-7143; *Bacillus globisporus* C11, FERM BP-7144; *Bacillus globisporus* N75, FERM BP-7591; and mutants thereof.

11. (Original) The process of claim 9, wherein said microorganism of the genus *Arthrobacter* is one selected from the group consisting of *Arthrobacter globiformis* A19, FERM BP-7590; and mutant thereof.

12. (Currently Amended) A method of α -glucosyl-transferring reaction, which comprises a step of contacting the purified α -isomaltosylglucosaccharide-forming enzyme of claim 1, ~~er-48,~~ 52, or 53 with a solution comprising a saccharide having a glucose polymerization degree of at least two and having the α -1,4 glucosidic linkage as a linkage at the non-reducing end.

13. (Currently Amended) The method of claim 12, wherein a saccharide-transferred product is formed by the α -glucosyl-transferring reaction in the presence of one or more acceptors selected from the group consisting of D-glucose, D-xylose, L-xylose, D-galactose, D-fructose, D-mannose, D-arabinose, D-fucose, D-psicose, L-sorbose, methyl- β -glucopyranoside, methyl- α -glucopyranoside, N-acetylglucosamine, trehalose, isomaltose, isomaltotriose, cellobiose, gentibiose, glycerol, maltitol, lactose, sucrose, and L-ascorbic acid.

14. (Currently Amended) A method for forming α -isomaltosyl- glucosaccharide, which comprises a step of contacting the purified α -isomaltosylgluco-saccharide-forming

enzyme of claims 1, ~~ex-48~~, 52, or 53 with a solution, comprising a saccharide having a glucose polymerization degree of at least two and having the α -1,4 glucosidic linkage as a linkage at the non-reducing end, to effect α -glucosyl-transferring reaction.

15. (Original) The method of claim 14, wherein said saccharide is one selected from the group consisting of maltooligosaccharides, maltodextrins, amyloextrins, amyloses, amylopectins, soluble starches, liquefied starches, and glycogens.

Claims 16 - 45. (Cancelled)

46. (Currently Amended) A biologically pure culture containing the α -isomaltosylglucosaccharide-forming enzyme of claims 1, ~~ex-48~~, 52, or 53.

47. (Currently Amended) The purified α -isomaltosylglucosaccharide-forming enzyme of claim 1, 52, or 53 wherein said enzyme has a partial amino acid sequence of SEQ ID NO:1 or SEQ ID NO:11.

48. (Currently Amended) A purified α -isomaltosylglucosaccharide-forming enzyme which forms a saccharide having a glucose polymerization degree of at least three and having both an α -1,6-glucosidic linkage as a linkage at the non-reducing end and an α -1,4-glucosidic linkage other than the linkage at the non-reducing end, by catalyzing α -glucosyl transfer from a saccharide having a glucose polymerization degree of at least two and having an α -1,4-glucosidic linkage as a linkage at the non-reducing end; said enzyme being obtainable from a microorganism of the genus *Arthrobacter* selected from the group consisting of *Arthrobacter*

globiformis A19, FERM BP-7590, and mutants thereof and having the following physicochemical properties:

- a. Molecular weight about $94,000 \pm 20,000$ Daltons on SDS-PAGE;
- b. Isoelectric point (pI) about 4.3 ± 0.5 on isoelectrophoresis using ampholine;
- c. Optimum temperature
 - (i). About 60°C when incubated at a pH of 8.4 for 60 minutes;
 - (ii). About 65°C when incubated at a pH of 8.4 for 60 minutes in the presence of 1 mM Ca^{2+} ;
- d. Optimum pH about 8.4 when incubated at 35°C for 60 minutes;
- e. Thermal stability
 - (i). Stable up to a temperature of about 55°C when incubated at a pH of 8.0 for 60 minutes;
 - (ii). Stable up to a temperature of about 60°C when incubated at a pH of 8.0 for 60 minutes in the presence of 1 mM Ca^{2+} ; and
- f. pH stability stable at pHs of about 5.0 to 9.0 when incubated at 4°C for 24 hours.

49. (Cancelled)

50. (Previously Presented) The α -isomaltosylglucosaccharide-forming enzyme of claim 48 wherein the enzyme has a partial amino acid sequence of SEQ ID NO:18.

51. (Currently Amended) The α -isomaltosylglucosaccharide-forming enzyme of claim 1 ~~or~~ 48, 52, or 53 which is ~~substantially~~ incapable of forming dextran,

inhibited by EDTA, and stabilized and/or activated by Ca^{2+} and Mn^{2+} .

52. (New) A purified α -isomaltosyl-glucosaccharide-forming enzyme which forms a saccharide having a degree of glucose polymerization of at least three and having both the α -1,6 glucosidic linkage as a linkage at the non-reducing end and the α -1,4 glucosidic linkage other than the linkage at the non-reducing end, by catalyzing the α -glucosyl-transfer from a saccharide having a degree of glucose polymerization of at least two and having the α -glucosidic linkage as a linkage at the non-reducing end; said enzyme being obtained from a microorganism of the genus *Bacillus* selected from the group consisting of *Bacillus globisporus* C11, FERM BP-7144 and mutants thereof, said enzyme having the following physicochemical properties:

- a. Molecular weight
About $137,000 \pm 20,000$ Daltons on SDS-PAGE;
- b. Isoelectric point (pI)
About 5.2 ± 0.5 on isoelectrophoresis using ampholine;
- c. Optimum temperature
 - (i) about 45°C when incubated at a pH of 6.0 for 60 minutes;
 - (ii) about 50°C when incubated at a pH of 6.0 for 60 minutes in the presence of 1 mM Ca^{2+} ;
- d. Optimum pH about 6.0 when incubated at 35°C for 60 minutes;
- e. Thermal stability
 - (i) Stable up to a temperature of about 40°C when incubated at a pH of 6.0 for 60 minutes;

(ii) Stable up to a temperature of about 45°C
when incubated at a pH of 6.0 for 60
minutes in the presence of 1mM Ca²⁺;

f. pH stability

Stable at pHs of about 5.0 to about 10 when
incubated at 4°C for 24 hours.

53. (New) A purified α -isomaltosyl-glucosaccharide-forming enzyme which forms a saccharide having a degree of glucose polymerization of at least three and having both the α -1,6 glucosidic linkage as a linkage at the non-reducing end and the α -1,4 glucosidic linkage other than the linkage at the non-reducing end by catalyzing α -glucosyl-transfer from a saccharide having a degree of glucose polymerization of at least two and having the α -glucosidic linkage as a linkage at the non-reducing end; said enzyme being obtained from a microorganism of the genus *Bacillus* selected from the group consisting of *Bacillus globisporus* N75, FERM BP-7591 and mutants thereof, said enzyme having the following physicochemical properties:

a. Molecular weight

About 136,000 \pm 20,000 Daltons on SDS-PAGE;

b. Isoelectric point (pI)

About 7.3 \pm 0.5 on isoelectrophoresis using
ampholine;

c. Optimum temperature

(i) About 50°C when incubated at a pH of 6.0
for 60 minutes;

(ii) About 55°C when incubated at a pH of 6.0
for 60 minutes in the presence of 1 mM Ca²⁺;

d. Optimum pH about 6.0 when incubated at 35°C for
60 minutes;

- e. Thermal stability
 - (i) Stable up to a temperature of about 45°C when incubated at a pH of 6.0 for 60 minutes;
 - (ii) Stable up to a temperature of about 50°C when incubated at a pH of 6.0 for 60 minutes in the presence of 1mM Ca²⁺;
- f. pH stability
 - Stable at pHs of about 5.0 to about 9.0 when incubated at 4°C for 24 hours.